SHORT COMMUNICATION

AMENABILITY OF THE SUGARCANE VARIETY 2005 T 16 TO SHOOT TIP CULTURE

B. Jagadeesh, M. Hemanth Kumar*, M. Reddi Shekhar and P. Sudhakar

Abstract

The protocol for regeneration of plantlets through in vitro culture using shoot tip was established for a pre-released early maturing sugarcane variety, 2005T16. Inoculation of shoot tip explants, surface sterilized with 10% sodium hypochlorite for 20 min on MS medium containing 3.0 mg l^{-1} BAP, 2.0 mg l^{-1} IAA and 2.0 mg l^{-1} kinetin led to axillary bud initiation and establishment. The highest multiple-shoot induction of 88.89% frequency within 12.3 days was also observed on the same medium with production of 12.3 multiple-shoots per axillary shoot. The best rooting of 77.78% and 6.8 roots per shoot having a mean length of 3.76 cm were recorded for the shootlets on half strength MS medium supplemented with 6 mg l^{-1} NAA. During acclimatization the highest survival percentage was observed in vermicompost: soil: sand mixture in 1:1:1 ratio.

***Key words**: Sugarcane, micropropagation, shoot induction, root induction*

Achieving the target of 100 t ha^{-1} productivity in sugarcane still remains as an objective to be fulfilled. Even though the released varieties have a genetic potential of 120 t ha^{-1}, varietal decline within a few cycles of vegetative propagation in the farmers' fields has been a major bottleneck for achieving the production potential. Availability of healthy seed is a prerequisite to sustainable cane production. Tissue culture techniques have been widely used for large-scale micropropagation as they can effectively reduce the time period between selection and commercial release of new sugarcane varieties (Lorenzo *et al*., 2001). Micropropagation is currently the only realistic means of achieving rapid, large-scale production of disease-free seed canes of newly developed varieties in order to speed up breeding and commercialization process in sugarcane. In contrast to conventional method where one bud produces 4-5 shoots, tissue culture, if estimated conservatively, can produce around 10,000 identical plants from a single bud in about 3-4 months (Lee, 1987). Plantlets developed from shoot tip are true to type without any variation from the mother plant (Ali and Afghan, 2001). Shoot tip explants offer greater multiplication rate along with virus elimination (Victoria and Guzman, 1993). In order to rapidly multiply a pre-release early sugarcane variety, 2005T16, developed at Agricultural Research Station, Perumallapalle, micropropagation protocol by using shoot tip explant was standardised.

The tops of the pre-release early sugarcane variety 2005T16 were collected from 7-8 month old plants maintained in mother nursery at Agricultural Research Station, Perumallapalle, and explants of 4-5 cm were dissected out. The explants were washed thoroughly under running tap water for 20-30 min, treated with 10% sodium hypochlorite for 20 minutes and finally washed 3-5 times with sterile distilled water. Shoot tips of 1.0-1.5 cm length were dissected before inoculation on MS medium supplemented with different concentrations and combinations of BAP, Kin, NAA and IAA for axillary shoot induction from shoot tip. Treatment combinations that were used for the axillary shoot induction and their establishment were also used for

B. Jagadeesh, M. Hemanth Kumar, M. Reddi Shekhar and P. Sudhakar
Agricultural Research Station, Perumallapalle,
Tirupati 517505, Andhra Pradesh, India
email: hemanthangrau@gmail.com

multiple-shoot induction. Fully grown multi-shoots were transferred to half strength and full strength MS medium supplemented with different concentrations of IBA (2-4 mg l^{-1}) and NAA (4-6 mg l^{-1}), both individually and in combinations. The pH of the medium was adjusted to 5.8 before adding agar and prior to autoclaving for 20 min at 120^0C and 15 lbs psi pressure. All the cultures inoculated in culture bottles containing medium with 30 gm l^{-1} sugar were incubated in a culture room with 16 h photoperiod, 25 ± 3^0C temperature and 70-80% RH. Each treatment consisted of eight replicates repeated thrice for establishment and six replicates repeated thrice for multiplication and rooting. Coconut peat, vermicompost, pressmud, soil and sand were used singly and in combinations of 1:1:1 for acclimatization and hardening. The percentage data recorded for various parameters were subjected to arc sine transformation and statistical analysis as suggested by Gomez and Gomez (1984).

Surface sterilization of explants with 10% sodium hypochlorite for 20 min in the present study was proved to be effective, as explants contamination after inoculation was only 16.7%. Among the different treatment combinations and concentrations of hormones used for axillary shoot induction and establishment, MS medium supplemented with 3 mg l^{-1} BAP + 2 mg l^{-1} IAA + 2 mg l^{-1} Kinetin recorded a maximum of 77.5% explant establishment with axillary shoots of 3.8 per explant and a mean height of 4.3 cm in 38.6 days. Significant differences were observed among the treatments. IAA was found to be better for axillary shoot production and establishment than NAA even though it is heat liable in nature. Young tissues had more cytokinin content than the older ones (George 1993) and conversion of cytokinin nucleotides to nucleosides stimulated macroscopic changes in buds thus promoting bud opening and increasing fresh and dry masses (Toteva and Stoyanova, 2003).

Shoot formation was highly influenced by the type of growth regulators and their concentrations used in the investigation. Among the different concentrations tested, the highest multi-shoot initiation was observed on MS medium supplemented with 3 mg l^{-1} BAP + 2 mg l^{-1} IAA + 2 mg l^{-1} Kinetin. In this combination, 88.89% of axillary shoots induced multi shoots within 12.3 days with 12.3 multi shoots produced per shoot and a mean length of 4.3 cm obtained within 20 days. The results of the present study confirmed that high level of cytokinin and a low level of auxin were essential for differentiation of adventitious shoots in sugarcane. Combinations of auxins and cytokinins rather than cytokinins alone gave good results supporting the earlier observation that their combination was essential for shoot regeneration (Baksha et al. 2002). The higher and lower concentrations did not show better shoot multiplication in this study. As reported by Flores and Tobin (1988), the primary mode of action of plant growth regulators involves binding of active substances to specific receptor molecule which bind either on cell surface or within the cytoplasm. The concentration of receptors to target tissues determined the response potential. Bud formation begins with an asymmetric division of target cell, which is located next to several cells away from the point of contact of media and tissue. This step is initiated by cytokinin that would bind to an unidentified receptor within the target cell and its further development requires continuous presence of cytokinins (Saunders and Helper, 1982).

Different concentrations and combinations of NAA and IBA were used with full strength and half strength MS media to initiate adventitious roots. Half strength MS medium was more responsive than full strength MS medium and NAA was better than IBA used either alone or in combination with other hormones. Half strength MS media supplemented with 6 mg l^{-1} NAA was found to be the best combination with 77.78% of shoots inducing roots within 14.3 days of inoculation of multiple-shoots on to rooting medium. The highest number of roots of 6.8 per shoot with mean length of 3.76 cm was obtained within 35 days. Similar results were obtained by Mamun et al. (2004). Among the various hardening mixtures evaluated, the treatment combination of vermicompost : soil : sand (1:1:1) recorded the highest survival percentage of 75% followed by the treatment combination of pressmud : soil : sand (1:1:1) which recorded 50% survival. Significant differences were observed among the treatments.

Thus the shoot tip explant of 2005T16 can be well established with full strength solid MS medium supplemented with 3.0 mg l^{-1} BAP, 2.0 mg l^{-1} IAA

and 2.0 mg l^{-1} Kinetin. The established explants can be efficiently subcultured and multiplied on the same medium. Rooting can best be achieved in half strength solid MS medium supplemented with 6.0 mg l^{-1} NAA alone. The plantlets with well developed shoots and roots were acclimatized on hardening mixture of vermicompost, soil and sand in 1:1:1 ratio.

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